# APPARATUS COMPRISING A REAGENT ATOMIZATION AND DELIVERY SYSTEM

## Field of the Invention

[0001] The present invention relates generally to dispensing very small volumes of liquid. More particularly, the invention relates to uniformly and simultaneously dispensing micro-liter volumes of liquid into small discrete regions on a specimen plate for triggering chemical/physiological reactions.

## Background of the Invention

[0002] In assay screening, a large number of cellular events (*e.g.*, calcium flux, *etc.*), physiological and/or molecular events (*e.g.*, chemical reactions, *etc.*) are monitored and analyzed. These events, hereinafter referred to as "target events," are usually carried out in parallel in an array of deposits on specimen plates (*e.g.*, slides, multi-well plates, *etc.*). The deposits comprise one or more reagents, cellular material, *etc.*

[0003] Due to the large number of target events taking place, time-consuming methods that directly examine each deposit, such as by microscopic examination, are unsuitable for data acquisition. Rather, a "snap shot" of the full array on the specimen plate is advantageously taken via visible spectrum or infrared spectrum imaging systems.

[0004] Two common visible-spectrum imaging processes are fluorescence imaging and luminescence imaging. In fluorescence imaging, when a target event occurs, a detection reagent in the deposit emits light (*i.e.*, fluoresces) when excited by an appropriate excitation source (*e.g.*, ultraviolet light). The detection reagent is chosen for its ability to interact (*e.g.*, bind, *etc.*) with a target or to respond to a specific stimulus that is present only if the target event occurs. The emitted light, which provides qualitative and/or quantitative information about the event, is captured and converted to electrical signals using, for example, a charge coupled device ("CCD"). The CCD comprises an array of thousands of sensor cells that are capable of receiving radiation from multiple samples at the same time. The signals are analyzed, via suitable processing electronics/software, to recover information concerning the event. Luminescent imaging (chemi- or bio-) is similar to fluorescence imaging, except that excitation radiation is not required.

[0005] FIG. 1 depicts a simplified schematic of a typical imaging device **100** for fluorescence imaging. Imaging device **100** includes cooled CCD camera **102**, emission filter **104**, optics **106**,

filter wheel **108** and illumination source **110**, interrelated as shown. Some other elements that are part of, or otherwise associated with imaging device **100** but that are not shown in FIG. 1 include a camera control unit, a computer with analysis software, a specimen positioner and a liquid dispenser.

**[0006]** In operation, excitation radiation **112** from illumination source **110** is delivered to specimen plate **114** containing a plurality of deposits. Excitation radiation **112** is delivered, for a pre-determined period of time, toward a selected deposit, group of deposits or the entire plate of deposits. At the end of the time period, the delivery of excitation radiation **112** ceases and a response occurs (*i.e.*, light **116-1, 116-2** is emitted) and is detected. In a process called time resolved fluorescence ("TRF"), after excitation radiation **112** ceases, the response is monitored after a specific delay of a few milliseconds.

**[0007]** Thermal or infrared spectrum imaging is an alternative to visible spectrum imaging. The principle underlying infrared spectrum imaging is that all chemical reactions and physiological processes are accompanied by a change in energy (*i.e.*, heat is absorbed or released), and this energy change can be monitored/measured to obtain useful information about the reactions/processes that are taking place.

**[0008]** FIG. 2 depicts a simplified schematic of an infrared imaging system disclosed in published PCT application WO 99/60630 for monitoring physiological and molecular events. Imaging system **200** comprises an infrared camera **218**, including optics **220**, that is spaced apart (*i.e.*, the lens has a 6 centimeter focal length) from target **222** (*e.g.*, a specimen plate having deposits — reagents, cellular or non-cellular material — arrayed thereon, *etc.*). Target **222** is contained within isothermal chamber **224** that reduces temperature variations. The infrared camera monitors radiated heat production from target **222** and images are recorded by central processing unit **226** for data capture and analysis. WO 99/60630 is incorporated by reference herein.

**[0009]** Infrared camera **218** advantageously provides a thermal image of the entire scene. That is, if a micro-well plate with its two-dimensional array of wells is being monitored, a thermal image of *each* well is obtained substantially simultaneously. This is best accomplished using a focal plane array or "staring" array.

**[0010]** A focal plane array ("**FPA**") **328**, depicted in FIG. 3, is a monolithic microelectronic device that incorporates thousands of sensing elements **330** that continuously receive IR radiation, capturing an image of the entire scene. FPA-based infrared cameras include a single

monolithic FPA detector and optics. Since an FPA-based infrared camera captures an image of all samples simultaneously, it supports ratiometric analysis.

**[0011]** For both visible spectrum and infrared spectrum imaging systems and methods, it is advantageous if not necessary for the reagents, *etc.*, that participate in the reactions being monitored to be delivered substantially simultaneously to locations on the specimen plate. Simultaneous delivery ensures that the reactions occurring at various locations (*e.g.*, each well, *etc.*) occur at the same time.

**[0012]** Simultaneous delivery is particularly important for time dependent assays (*e.g.*, TRF). Furthermore, simultaneous delivery of reagent is very important for thermal imaging processes that are ratiometric and comparative. Also, to the extent that reagents evaporate, such evaporation is less problematic when reagent is delivered in parallel (simultaneously) rather than sequentially.

**[0013]** Unfortunately, most prior-art liquid delivery systems cannot deliver liquid simultaneously to all wells in a multi-well plate. The art would therefore benefit from a dispenser and system capable of dispensing sub-micro liter volumes of liquid substantially simultaneously to all desired locations on a specimen plate.

## Summary of the Invention

**[0014]** An apparatus comprising a reagent atomization and delivery system is disclosed. In the illustrative embodiments, the reagent atomization and delivery system is used to uniformly and simultaneously dispense micro-liter volumes of liquid into a plurality of small cavities, for example, such as are defined in a multi-well plate. Once dispensed, the liquid triggers chemical/physiological reactions that are then advantageously subjected to imaging analysis (*e.g.*, visible-spectrum imaging, infrared spectrum imaging, *etc.*).

**[0015]** In some embodiments in accordance with the principles of the invention, a reagent atomization and delivery system includes an atomizer and a mask. The atomizer delivers a spray of atomized reagent toward a specimen plate. The velocity of liquid droplets that comprise the atomized reagent is advantageously less than about 3 meters per second, and preferably less than about 1 meter per second. The mask, which has a plurality of openings therein, is disposed between the specimen plate and the atomizer. The openings restrict the passage of atomized reagent to the specimen plate, such that liquid is received only at desired regions on the specimen

plate. That is, reagent is received within the wells, rather than the region between the wells, on a multi-well plate, for example.

**[0016]** In another embodiment, a voltage or electrical potential is applied to various elements of the reagent atomization and delivery system to electrostatically focus and direct the atomized reagent to desired locations on the specimen plate.

**[0017]** The reagent atomization and delivery system advantageously comprises an ultrasonic atomizer, which consists of an ultrasonic power supply for generating high frequency energy, a converter for converting the high frequency electrical energy to vibrational energy, and an atomizing nozzle for amplifying the vibrations and using them to atomize reagent that is delivered to the nozzle.

## Brief Description of the Drawings

**[0018]** **FIG. 1** depicts a simplified schematic of a typical prior art fluorescence imaging system.

**[0019]** **FIG. 2** depicts a simplified schematic of a prior art infrared-spectrum imaging system.

**[0020]** **FIG. 3** depicts a focal plane array, such as can be used in the infrared-spectrum imaging system of FIG. 2.

**[0021]** **FIG. 4** depicts an apparatus comprising an imaging system and an fluid atomizing and delivery system in accordance with an illustrative embodiment of the present invention.

**[0022]** **FIG. 5** depicts an ultrasonic atomizer for use in conjunction with the invention.

**[0023]** **FIG. 6** depicts an atomizing nozzle delivering atomizer reagent to a specimen plate in accordance with the principles of the invention.

**[0024]** **FIG. 7** depicts a mask sandwiched between an atomizing nozzle and a specimen plate.

**[0025]** **FIG. 8** depicts the atomizing nozzle, mask and specimen plate of FIG. 7 arranged to electrostatically focus atomized reagent.

**[0026]** **FIG. 9** depicts a visible spectrum imaging system disclosed in U.S. Pat. App. No. ______ (Atty Dkt. PH1093).

**[0027]** **FIG. 10** depicts an infrared spectrum imaging system disclosed in U.S. Pat. App. No. ___ (Atty Dkt. PH1094).

[0028] **FIG. 11** depicts a method in accordance with the illustrative embodiment of the present invention.

## Detailed Description

[0029] The terms listed below are given the following specific definitions for the purposes of this specification.

[0030] "**Atomized liquid**" is a liquid that has been broken down into micro-droplets having a diameter less than about 500 microns. For the purposes of this specification, the word "atomized" is synonymous with the word "nebulized."

[0031] "**Atomizer**" is a device that creates an atomized liquid.

[0032] "**Infrared Spectrum Radiation**" means radiation having a wavelength within a range of about 780 nanometers to about 1 millimeter.

[0033] "**Reagents**" means cellular material, non-cellular material and/or chemicals. Generally, the term "reagent" means anything that is a reactant, solvent or otherwise participates in target events.

[0034] "**Specimen plate**" means a plate on which reagent(s) are disposed. The term "specimen plate" includes multi-well (*e.g.*, micro-titer) plates. Such plates have a plurality of wells (96-well, 384-well, 1536-wells are typical) that are organized in a two dimensional array. The term "specimen plate" also refers to a glass or plastic slide that does not have wells, upon which reagents are deposited in large two-dimensional arrays.

[0035] "**Target Events**" means cellular, physiological and/or molecular events, such as, for example, calcium flux, chemical reactions, *etc.*

[0036] "**Visible Spectrum Radiation**" means radiation having a wavelength in the visible range, which is about 390 nanometers to about 780 nanometers.

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[0037] FIG. 4 depicts apparatus **400** in accordance with the illustrative embodiment of the invention. Apparatus **400** comprises imaging system **432**, reagent atomization and delivery system **436** and positioner **440**. The positioner shuttles specimen plate **442** between first position **444** and second position **446**. Positioner **440** can be any one of a variety of mechanisms known in the art for positioning, such as, without limitation, a motorized positioning stage.

[0038] In first position **444**, specimen plate **442** is operatively engaged to reagent atomization and delivery system **436**. That is, specimen plate **442** is positioned to receive reagent in the form of an atomized liquid from atomizer **438**. In second position **446**, specimen plate **442** is operatively engaged to imaging system **432**. That is, specimen plate **442** is positioned such that detector **434** of imaging system **432** can detect target events occurring thereon.

[0039] The atomized liquid delivered by atomizer **438** consists of micro-droplets having a diameter of less than about 500 microns. The average size of micro-droplet is advantageously in a range of about 25 to 100 microns. As is well known, a variety of different methods exist for atomizing liquid. And while such methods can suitably be used, atomizer **438** is advantageously an ultrasonic atomizer.

[0040] In contrast to most other types of atomizers, ultrasonic atomizers do not rely solely on pressure and high-velocity motion to shear a fluid into micro-droplets. Specifically, ultrasonic atomizers use ultrasonic vibrational energy to enhance atomization. Consequently, the velocity of the micro-droplets generated by ultrasonic atomizers is quite low (*i.e.*, typically less than 0.5 meters/sec) in comparison with the 5 to 20 meters/second velocities typical for pressurized-type atomizers. The low velocity of droplets produced by ultrasonic atomizers advantageously reduces the tendency of the droplets to bounce off a receiving surface, such as specimen plate **442**. The velocity of the droplets is advantageously about 3 meters/second or less, and preferably less than about 1 meter per second.

[0041] Apparatus **400** is depicted as being contained within environmental enclosure **447**. It may not be practical, in all embodiments, to have imaging system **432** within environmental enclosure **447** due to the specific configuration of the imaging system. But in all embodiments, reagent atomizer and delivery system **436** is should be contained with environmental enclosure **447** since the fine, low velocity spray delivered by atomizer **438** is readily disrupted by air currents, *etc.*

[0042] FIG. 5 depicts ultrasonic atomizer **438**, which is readily available commercially from Cole-Parmer Instrument Co. (Vernon Hills, Ill.); Lechler GmbH (Germany); Sonics and Materials Inc. (Danbury, Conn), and others. Ultrasonic atomizer **438** comprises ultrasonic power supply **548**, converter **552** and atomizing nozzle **554**, interrelated as shown. Ultrasonic power supply **548** converts 50/60 Hz voltage to high frequency electrical energy at 20 kHz or more. This high frequency signal is delivered, over line **550**, to converter **552**. The converter contains a piezoelectric transducer that changes the high frequency electrical energy to ultrasonic mechanical vibrations. The ultrasonic vibrations are intensified by nozzle **554**, which has large

diameter portion **556** and small diameter portion **558**. Reagent is delivered to nozzle **554** at liquid inlet **559**.

[0043] Atomizing nozzle **554** operates at a particular frequency that is determined by the frequency generator and optimized by nozzle geometry and the resonant frequency of the thereof. Nozzle **554** must be an integral number of half-wavelengths in length to produce standing waves, which are required to produce atomization. The vibrations are focused at orifice **560** of nozzle **554**, which is where atomization takes place. The amplitude of the standing wave is much greater near orifice **560** due to the amplification of motion provided by the step transition in diameter between large diameter portion **556** and small diameter portion **558**.

[0044] The diameter $d$ <meters> of atomized reagent droplets is related to the surface tension $\phi$ <Newtons/meter>, density $\rho$ <kilogram/cubic meter> and frequency $f$ <Hertz> of the reagent as follows:

$$[1] \qquad d = [\phi/(\rho f^2)]^{1/3}$$

[0045] With reference to FIG. 4, specimen plate **442** is positioned, by positioner **440**, beneath atomizing nozzle **554** (*i.e.*, in first position **446**) to receive reagent in the form of an atomized liquid. As depicted in FIG. 6, atomized reagent **662** is delivered from atomizing nozzle **554** in such a way that the spray just covers specimen plate **442**. Coverage is a function of the spray angle and the distance of the nozzle orifice **560** to specimen plate **442**. Typically, a spray angle between about 30 to 60 degrees and a distance within the range of about 7.5 to 15 centimeters should provide the requisite coverage for a standard-sized multi-well plate, *etc.* Using atomizer **438** in accordance with the principles of the invention should result in a coefficient of variation ("cv") of less than about twelve percent for reagent delivery across specimen plate **442**.

[0046] It is appreciated that in the embodiment depicted in FIG. 6, atomized reagent **662** completely covers the surface of specimen plate **442**. Consequently, for most applications, specimen plate **442** must be a multi-well plate, not a slide or other type of flat plate.

[0047] FIG. 7A depicts a variation of reagent atomization and delivery system **436** that addresses the problem of surface contamination of plate **442**. In particular, in the variation depicted in FIG. 7, mask **764** is disposed between atomization nozzle **554** and specimen plate **442**. Mask **764** defines a plurality of openings **766** that align with the intended deposition sites (*e.g.*, wells, *etc.*) of reagent on specimen plate **442**. As illustrated in FIG. 7B, mask **764** substantially reduces the incidence of over spraying reagent beyond the intended deposition sites **767**. Thus, when mask **764** is used, a slide as well as a micro-well plate can be used as specimen plate **442**. Mask **764** is

wiped (*e.g.*, with teflon blades, *etc.*) to remove reagent after reagent delivery. Mask **764** can be formed, for example, from stainless steel or any material that does not react with the reagents being used and that is suitably machineable, *etc.*, to create openings **766**.

**[0048]** FIGS. 8A and 8B depict a further variant of reagent atomization and delivery system **436** wherein elements of the system are electrically charged to electrostatically focus atomized reagent to the intended deposition sites. As depicted in FIG. 8A, controlled voltage source **868** is electrically connected to atomization nozzle **554** such that the nozzle becomes an anode (positive charge) and is electrically connected to conductive (*e.g.*, metal, *etc.*) sub-plate **869** that is disposed under specimen plate **442**. Sub-plate **869** becomes a cathode (negative charge). Furthermore, controlled voltage source **868** is also electrically connected to mask **764** (which must be suitably conductive to that end) to place a slight positive charge thereon. As depicted in FIG. 8B, mask **764** functions as a grid that directs atomized reagent to the middle of all openings **766**. This further reduces the incidence of over spraying the intended reagent deposition sites **767** on specimen plate **442**.

**[0049]** To obtain even further improvements in the electrostatically focused delivery of reagent, small electrically-conductive probes or needles (*see*, FIG. 8B) can be disposed on sub-plate **869** and positionally correlated to the center of each desired reagent delivery site (*e.g.*, the center of each well in a multi-well plate) on specimen plate **442**. Since the probes are negatively charged, positively charged atomized reagent is attracted to the probes and, hence, directed toward the center of each reagent delivery site.

**[0050]** In accordance with the principles of the invention, in some variations of apparatus **400**, imaging system **432** is a visible spectrum imager. Suitable visible spectrum imaging systems include, for example, prior art fluorescence or luminescence imaging systems, such as imaging system **100** depicted in FIG. 1.

**[0051]** Further suitable visible spectrum imaging systems include those disclosed in "Method and Apparatus for Visible Spectrum Imaging," filed on ________ as U.S. Pat. App. No. ________ (Atty. Dkt. PH1093 filed on even date herewith) and incorporated by reference herein.

**[0052]** In contrast to conventional visible imaging systems, the imaging systems disclosed in U.S. Pat. App. No. ____ (Atty Dkt. PH1093) have a very small gap between the detector and the reagents on the specimen plate. A methodology for determining gap size is described U.S. Pat. App. No. _____ (Atty Dkt. PH1093). Furthermore, some of the imaging systems disclosed

therein do not use optics (*e.g.*, lenses, *etc.*) between the specimen plate and the detector to collimate or focus emitted light.

[0053] FIG. 9 depicts an illustrative embodiment of the imaging system disclosed in U.S. Pat. App. No. _____ (Atty Dkt. PH1093). The imaging system comprises specimen plate **442**, excitation radiation filter **970**, detector **972**, excitation radiation source **976** and signal processing electronics **980**, arranged as shown. When configured for luminescent imaging, the imaging system does not require excitation radiation source **976** and excitation radiation filter **970**.

[0054] In the illustrative imaging system depicted in FIG. 9, excitation radiation source **976** is disposed beneath specimen plate **442**, which is in turn disposed beneath excitation radiation filter **970**, which is in turn disposed beneath detector **972**. Many variations on this specific arrangement (*i.e.*, detector **972** above filter **970** above specimen plate **442**) are suitable for use in conjunction with the imaging system. Several of these variations are described in U.S. Pat. App. No. ___ (Atty Dkt. PH1093).

[0055] In use, specimen plate **442** has a plurality of reagents disposed thereon, which were delivered thereto by reagent atomization and delivery system **436**. Detector **972** detects visible-spectrum light that is generated either directly (*i.e.*, via luminescence) or indirectly (*i.e.*, via fluorescence) from target events that are triggered by the reagent on specimen plate **442**. In some embodiments, detector **972** is a CCD camera, well known in the art, that comprises a number of sensor cells **974**.

[0056] When exposed to electromagnetic radiation having a wavelength that is within its operating range, detector **972** generates electrical signals $\mathbf{978}_{i,\, i=1,n}$. Signals $\mathbf{978}_{i,\, i=1,n}$ are then delivered to signal processing electronics **980** for analysis. Signal processing electronics **980** include analog-to-digital ("A/D") converter **982** and data processing system **986**. A/D converter **982** converts analog signals $\mathbf{978}_{i,\, i=1,n}$ to digital signals **984** suitable for processing by data processing system **986**.

[0057] Data processing system **986** comprises input/output ("I/O") **988**, processor **990**, and data storage device **992**. I/O **988** includes machine interfaces (*e.g.*, input and output ports, *etc.*) and human interfaces (*e.g.*, keyboard, monitor, *etc.*). Data storage device **992** is advantageously a non-volatile memory. Processor **990** is capable of storing data in and retrieving data from data storage device **992**, and is further capable of executing programs, such as analysis software **994**, that are stored in data storage device **992**, and of outputting data to I/O **988**. Data processing

should be fast enough and powerful enough to simultaneously monitor all wells. This is especially important for time resolved fluorescence ("TRF") imaging, as is known in the art.

[0058] In some additional variations of apparatus **400**, imaging system **432** is an infrared spectrum imager. Infrared imaging processes cannot reliably provide quantitative information about target events. This is because changes in emitted infrared radiation due to the occurrence of target events are quite minor in comparison with shifts in ambient temperature due to various mechanisms, including reagent evaporation, *etc*. Consequently, IR emissions are subject to too much background or "zero" line fluctuation noise for absolute measurements. In other words, the signal- to-noise ratio is too low. But useful ratiometric (*i.e.*, comparative) data can be obtained via infrared spectrum imaging.

[0059] Infrared spectrum imaging systems suitable for use in conjunction with invention include prior art infrared imaging systems, such as imaging system **200** depicted in FIG. 2. Further suitable infrared spectrum imaging systems include those disclosed in "Method and Apparatus for Infrared Spectrum Imaging," filed on _________ as U.S. Pat. App. No. ________ (Atty. Dkt. PH1094 filed on even date herewith) and incorporated by reference herein.

[0060] In contrast to conventional infrared imaging systems, the imaging systems disclosed in U.S. Pat. App. No. ____ (Atty Dkt. PH1094) have a very small gap between the detector and the reagents on the specimen plate. A methodology for determining gap size is described U.S. Pat. App. No. _____ (Atty Dkt. PH1094). Furthermore, some of the imaging systems disclosed therein do not use optics (*e.g.*, lenses, *etc.*) between the specimen plate and the detector to collimate or focus emitted light. FIG. 10 depicts an illustrative embodiment of the imaging system disclosed in U.S. Pat. App. No. _ (Atty Dkt. PH1094).

[0061] FIG. 10 depicts the infrared-spectrum imaging system disclosed in U.S. Pat. App. No. __ (Atty Dkt. PH1094). The imaging system comprises specimen plate **442**, detector **1002** and signal processing electronics **1008**, arranged as shown. In the illustrative imaging system depicted in FIG. 10, specimen plate **442** is disposed beneath detector **1002**. In a variation described in U.S. Pat. App. No. _______ (Atty Dkt. PH1094) that is suitable for use in apparatus **400** in accordance with the illustrative embodiment of the invention, specimen plate **442** is disposed above detector **1002**.

[0062] In use, specimen plate **442** has a plurality of reagents disposed thereon, as received by reagent atomization and delivery system **436**. Detector **1002** detects infrared-spectrum light that is generated from target events that are triggered by the reagent on specimen plate **442**. Infrared-

spectrum light detectors incorporate materials that exhibit a response (*e.g.*, generate an electrical signal) to some wavelengths of infrared radiation. To be suitable for use as the chemical component of an infrared detector, an infrared-responsive material must:

- exhibit sufficiently high sensitivity (*e.g.*, the ratio of electrical signal output to incident radiation power must be acceptable);
- exhibit sufficiently low internal noise (*i.e.*, due to molecular motion);
- exhibit a sufficiently linear response;
- respond (*i.e.*, how quickly the detector responds to changes in the level of infrared radiation) acceptably fast;
- have a sufficient bandwidth.

[0063] Illustrative materials that typically satisfy the above requirements, and that are cost effective for use, include (1) lead salts, such as PbS, PbSe and PbTe; (2) indium compounds, such as InSb, InAs and InGaAs; (3) formulations of HgCdTe; and (4) platinum silicide (PtSi). The choice of detector material is a function of application specifics, and those skilled in the art can select a suitable material in light of such specifics.

[0064] Detector **1002** is advantageously implemented as a focal plane array, well known in the art. A FPA comprises a regularly organized grouping of thousand of sensor elements **1004**. The radiation emitted from a particular region of plate **442** is received by only a small portion of the total of sensor elements **1004** comprising detector **1002**. Consequently, multiple groups of sensor elements **1004** are required to detect all of the target events that are occurring on plate **442**.

[0065] When exposed to infrared-spectrum radiation having a wavelength that is within its operating range, sensor elements **1004** generate an electrical response that is read-out in well known fashion. In this regard, FPAs are similar to the well-known CCDs. FPAs are commercially available from Sensors, Inc. of Princeton, NJ, among others.

[0066] The electrical responses from sensor elements **1004** are read-out and combined in known fashion to produce detector output signals $\mathbf{1006}_{i,\, i=1,n}$, which are delivered to signal processing electronics **1008** for analysis. Signal processing electronics **1008** include analog-to-digital converter **1010** and data processing system **1014**. Analog-to-digital ("**A/D**") converter **1010** converts analog signals $\mathbf{1006}_{i,\, i=1,n}$ to digital signals **1012** suitable for processing by data processing system **1014**. Data processing system **1014** is configured in the manner of data

processing system **986** described earlier in conjunction with the visible-spectrum imaging system depicted in FIG. 9.

[0067] As previously described, reagent atomization and delivery system **436** (FIG. 4) is advantageously contained within an environmental enclosure (*e.g.*, environmental enclosure **447**). To the extent that imaging system **432** is contained with environmental enclosure **447** as well, the enclosure should be opaque to the appropriate spectrum of radiation. That is, if imaging system is a visible-spectrum imaging system, enclosure **447** should be opaque to visible light. Similarly, if imaging system **432** is an infrared spectrum imaging system, enclosure **447** should opaque to IR. Since some materials, like polystyrene, are opaque to infrared only in certain wavelength ranges, environmental enclosure **447** advantageously comprises multiple layers of material, as necessary, to provide IR blocking over the appropriate range of wavelengths.

[0068] In some embodiments of the present invention, multiple atomizers **438** are present within reagent atomization and delivery system **436**, each for dispensing different reagents. For example, while a first atomizer adds the non-varying reagent, the additional atomizers can add, without limitation, accelerating reagents and/or quenching reagents and/or evaporation-reducing reagents, cooling agents, *etc*.

[0069] FIG. 11 depicts a method in accordance with the principles of the present invention. In accordance with operation **1102** of method **1100** (FIG. 11), reagent is atomized. As previously described, atomization is advantageously performed using ultrasonic vibration to form micron-sized droplets of reagent.

[0070] In operation **1104**, atomized reagent is delivered to a specimen plate. In some embodiments, operation **1102** further comprises the step of positioning said specimen plate in a first position to receive said atomized reagent. In some embodiments, operation **1104** further comprises passing the atomized reagent through a mask, such as mask **766**, as previously described (*see* FIG. 7B). In yet some additional embodiments, operation **1104** further comprises electrostatically focusing the reagent by applying a potential to various elements of reagent atomization and delivery system **436**, as previously described.

[0071] In accordance with operation **1106**, a target event that is triggered by said reagent is detected on said specimen plate. In some embodiments, operation **1106** further comprises the step of positioning said specimen plate in a second position to detect said target event. In some further embodiments, the step of detecting comprises detecting visible spectrum radiation. In some other embodiments, the step of detecting comprises detecting infrared spectrum radiation.

[0072] It is to be understood that the above-described embodiments are merely illustrative of the invention and that many variations may be devised by those skilled in the art without departing from the scope of the invention and from the principles disclosed herein. It is therefore intended that such variations be included within the scope of the following claims and their equivalents.